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PERSPECTIVES IN RENAL MEDICINE

Physiologic and molecular aspects of the Na^+:HCO_3^- cotransporter in health and disease processes

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Physiologic and molecular aspects of the Na^+:HCO_3^- cotransporter in health and disease processes. Approximately 80% of the filtered load of HCO_3^- is reabsorbed in the proximal tubule via a process of active acid secretion by the luminal membrane. The major mechanism for the transport of HCO_3^- across the basolateral membrane is via the electrogenic Na^+:$3HCO_3^-$ cotransporter (NBC). Recent molecular cloning experiments have identified the existence of three NBC isoforms (NBC-1, NBC-2, and NBC-3).[1] Functional and molecular studies indicate the presence of all three NBC isoforms in the kidney. All are presumed to mediate the cotransport of Na^+ and HCO_3^- under normal conditions and may be functionally altered in certain pathophysiologic states. Specifically, NBC-1 may be up-regulated in metabolic acidosis and potassium depletion and in response to glucocorticoid excess and may be down-regulated in response to HCO_3^- loading or alkalosis. Recent studies provide molecular evidence indicating the expression of NBC-1 in pancreatic duct cells. NBC is activated by cystic fibrosis transmembrane conductance regulator (CFTR) and plays an important role in HCO_3^- secretion in the agonist-stimulated state in pancreatic duct cells. The purpose of this review is to summarize recent functional and molecular studies on the regulation of NBCs in physiologic and pathophysiologic states. Possible signals responsible for the regulation of NBCs in these conditions are examined. Furthermore, the possible role of this transporter in acid-base disorders (such as proximal renal tubular acidosis) is discussed.

Proximal tubular acidification processes are primarily responsible for reabsorption of most of the bicarbonate present in glomerular filtrate. The vast bulk of this reabsorption occurs via *trans*-cellular coupling of the luminal Na^+/H^+ exchanger NHE-3 and H^+-ATPase [1–4] with the basolateral Na^+:HCO_3^- cotransporter (NBC) [5–8]. Although NBC was first described in the kidney proximal tubule, recent studies have shown its presence in numerous types of cells, including brain, liver, colon, cornea, heart, and lung [9–16], suggesting that this pathway plays an important role in mediating HCO_3^- transport in both epithelial as well as nonepithelial cells. Functional data support the presence of more than one NBC isoform, as judged by direction and stoichiometry of the transporter. In kidney proximal tubule, NBC activity leads to cell acidification, whereas in other tissues (liver and heart), its function leads to cell alkalinization. Furthermore, NBC has a stoichiometry of three equivalents of HCO_3^- per Na^+ ion in the kidney but shows a stoichiometry of 2 HCO_3^- per Na^+ in other tissues.

In addition to the NBC, a Na-dependent Cl^-/HCO_3^- exchanger is also expressed in kidney (and certain other tissues) and is shown to be involved in cell pH regulation [3, 5]. The Na-dependent Cl^-/HCO_3^- exchanger is an electroneutral transporter and mediates transport of Na^+ and HCO_3^- into the cell in exchange for cell Cl^- [3, 5]. This transporter is likely to be structurally related to NBC-1, based on functional data showing transport of both HCO_3^- and Na^+, and sensitivity to 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) [3, 5].

Although much information has been gathered on the molecular properties and gene regulation of N^+/H^+ exchanger (NHE) and anion exchanger (AE) isoforms, molecular properties of Na-coupled HCO_3^- transporters (gene structure and regulation) have not been characterized. This has been due to the lack of information on the genes encoding these transporters. Recent molecular cloning experiments have identified the presence of several NBC isoforms. These isoforms display distinct tissue distribution patterns and are differentially regulated in certain pathophysiologic states. Although characterization of NBC isoforms is still in its early stage, much information has been gathered about tissue-specific NBCs. The proximal tubule NBC has been characterized

[1] At the time this paper was under review, the cloning of a new NBC isoform, which is also called NBC-3, was reported (Pushkin A, Abuladze N, Lee I, Newman D, Hwang J, Kurtz I. *J Biol Chem* 274:16569–16575, 1999). We therefore have two distinct NBC-3 isoforms. To prevent any confusion, they are named kNBC-3 (reference 85) and mNBC-3 (Pushkin et al, see above). mNBC-3 is expressed only in myocytes and myocardium. kNBC-3 (reference 85) is the isoform expressed in kidney and other tissues. The term "NBC-3" used in this article refers to kNBC-3.

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with respect to electrogenicity and stoichiometry, ionic base species, cation and anion specificity, inhibitor profile, pH sensitivity, and functional and molecular regulation in acute and chronic states. These aspects are discussed in the following sections. Furthermore, we discuss the characterization of NBC isoforms and their regulation in pathophysiologic disorders.

ELECTROGENECITY, STOICHIOMETRY, AND IONIC BASE SPECIES

Kidney

Based on the electrochemical gradients of Na^+ and HCO_3^- across the basolateral membrane (BLM) of the kidney proximal tubule, there should be a net flux of HCO_3^- from blood to the proximal tubule cell if NBC carries one Na^+ for one HCO_3^-. However, in all studies to date, NBC has been found to be electrogenic and associated with a net flux of negative charge [5–16]. Boron and Boulpaep found that transport of HCO_3^- across the BLM of amphibian proximal tubule was associated with a net movement of negative charge in the same direction [17]. Similarly, Yoshitomi, Burckhardt and Fromter [18], and Alpern [19] showed that in mammalian proximal tubule cell, NBC is an electrogenic pathway and carries a net negative charge. Grassl and Aronson showed that in the presence of CO_2/HCO_3^- buffer and in the absence of an initial HCO_3^- gradient, Na^+ influx was stimulated in BLM vesicles when an inside positive membrane potential was imposed using an inward K^+ gradient and the K^+ ionophore valinomycin [20]. These observations indicate that the stoichiometry of the cotransport process must involve more than 1 HCO_3^- per Na^+. Knowledge of the precise stoichiometry is important for predicting the direction of net transport under physiologic and pathophysiologic conditions. The greater the HCO_3^- per Na^+ stoichiometry, and hence the greater the net negative charge movement per transport event, the more effectively the inside-negative membrane potential of the cell can drive the net exit of HCO_3^- against the Na^+ and HCO_3^- gradients. For example, recent measurements [18] indicate that the membrane potential would not be sufficient to drive net HCO_3^- efflux across the BLM of the proximal tubule cell under physiologic conditions if the stoichiometry of cotransport were only 2 HCO_3^-:Na^+. Two separate studies in perfused rat proximal tubule and in rabbit BLM vesicles showed that NBC has an apparent stoichiometry of 3 HCO_3^- per Na^+ ion [18, 21]. With such a stoichiometry, the inside-negative membrane potential, normally on the order of -60 mV, is sufficient to drive HCO_3^- exit against the inward concentration gradients of HCO_3^- and Na^+ that are present across the BLM of the intact proximal tubule cell.

It should be emphasized that although the results of these studies are consistent with a stoichiometry of 3 HCO_3^- per Na^+ ion, they are equally consistent with any transport process in which there is the net transfer of three equivalents of base and one Na. Thus, for example, the cotransport of Na^+ with 3 HCO_3^-, the cotransport of Na^+ with 2 HCO_3^- and 1 OH^- and/or cotransport of Na^+ with 1 HCO_3^- and 1 $CO_3^=$ are thermodynamically equivalent processes. The nature of the base species transported via NBC was studied by ^{22}Na influx method in BLM vesicles isolated from rabbit renal cortex [22]. The results showed that ^{22}Na influx was stimulated when [$CO_3^=$] was increased at constant [HCO_3^-], indicating the existence of a transport site for $CO_3^=$. The results further showed that the binding of HCO_3^- to a distinct site is essential for the binding of $CO_3^=$ to its site [22]. Based on these studies, it was concluded that the HCO_3^- exit across the BLM of the kidney proximal tubule occurs via a cotransport of $1Na^+$:$1CO_3^=$:$1HCO_3^-$ on separate distinct sites [22], consistent with a stoichiometry of three equivalents of base per Na [21].

Nonrenal tissues

NBC is expressed in several mammalian cell types, including epithelial as well as nonepithelial cells. A major difference with respect to the functional mode of the NBC in kidney and other tissues is its direction of transport. In kidney proximal tubule cells, this transporter mediates the exit of HCO_3^- from the cell to the blood [5–8], whereas in other epithelial cells and certain nonepithelial cells such as heart [14, 15] and liver [10, 11], this transporter mediates the entry of HCO_3^- from blood to the cell. As such, in the kidney proximal tubule, NBC functions as an acid loader, because its direction of transport leads to cell acidification. However, in heart or liver, NBC functions as an alkaline loader, because its direction of transport leads to cell alkalinization. Furthermore, the kidney NBC has a stoichiometry of three base equivalents per 1 Na, whereas the brain NBC has a stoichiometry of two base equivalents per Na [5–8]. Heart NBC is either electroneutral [14] or has a stoichiometry of 2 bases per Na [15], perhaps depending on the myocardial cell type.

Whether the difference in the direction of NBC movement in kidney and other tissues is due to differences in the membrane potential or cell ionic compositions in these tissues or whether it suggests the presence of other isoforms of this transporter has been the subject of intense speculation. Recent molecular studies illustrate that the proximal tubule NBC [23–27] is distinct from the cardiac NBC, indicating that the opposite functional modes of cotransport (influx in the cardiac cells and efflux in the kidney cells) are likely because of the presence of two different isoforms in these tissues. A comparison of the studies in kidney and pancreas, however, indicates that the same NBC isoform can work in oppo-

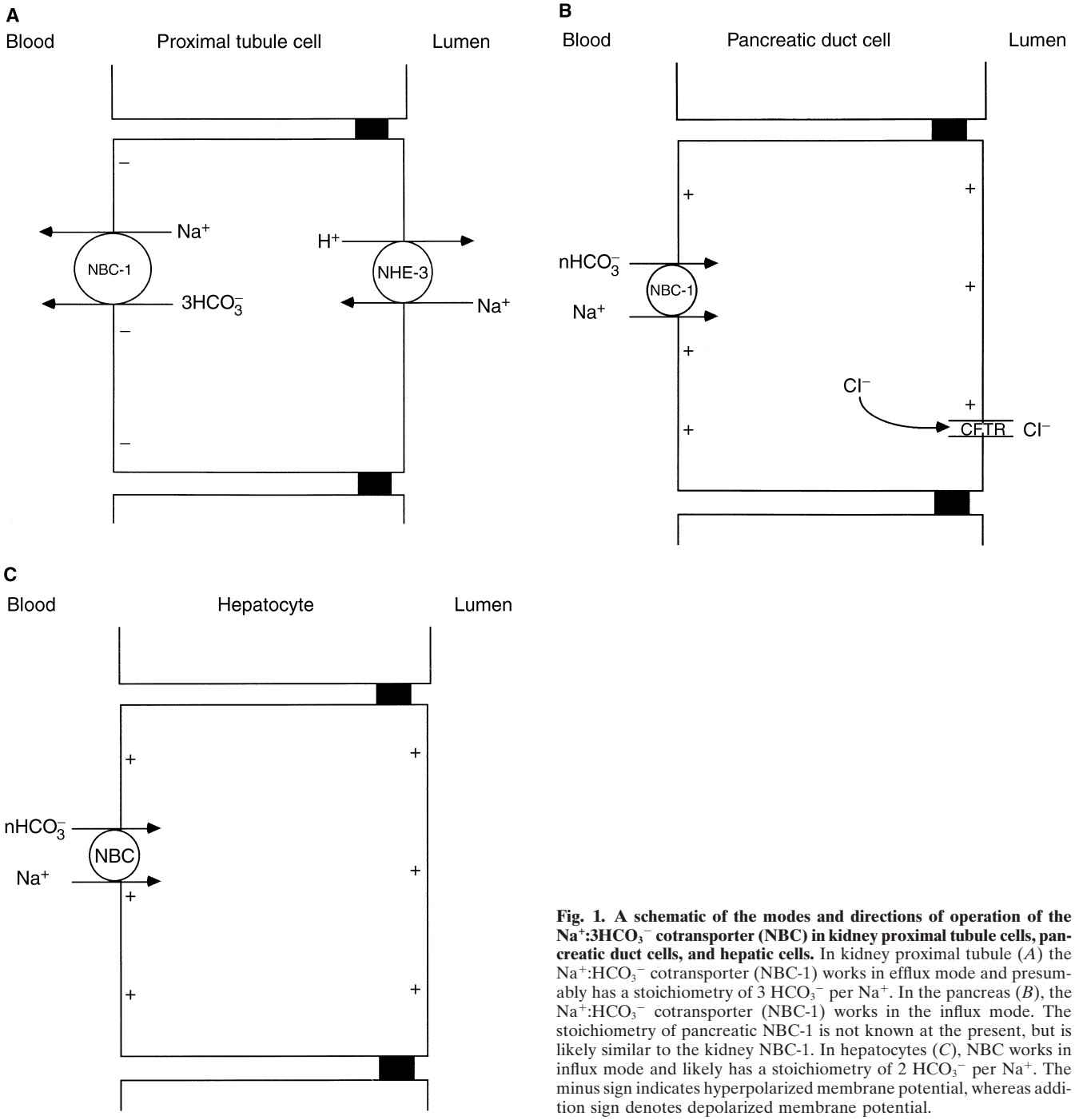

Fig. 1. A schematic of the modes and directions of operation of the Na^+:3HCO_3^- cotransporter (NBC) in kidney proximal tubule cells, pancreatic duct cells, and hepatic cells. In kidney proximal tubule (*A*) the Na^+:HCO_3^- cotransporter (NBC-1) works in efflux mode and presumably has a stoichiometry of 3 HCO_3^- per Na^+. In the pancreas (*B*), the Na^+:HCO_3^- cotransporter (NBC-1) works in the influx mode. The stoichiometry of pancreatic NBC-1 is not known at the present, but is likely similar to the kidney NBC-1. In hepatocytes (*C*), NBC works in influx mode and likely has a stoichiometry of 2 HCO_3^- per Na^+. The minus sign indicates hyperpolarized membrane potential, whereas addition sign denotes depolarized membrane potential.

site directions in two different tissues. For example, NBC-1 is expressed in both kidney proximal tubule [25–27] and pancreatic duct cells [28, 29]; however, it works in the influx mode in the pancreatic duct cells [29] but operates in the efflux mode in the kidney proximal tubule [5–8]. This is due to a depolarized membrane potential in the pancreatic duct cells, which results from CFTR activation with subsequent Cl^- secretion [30]. The schematic diagram in Figure 1 illustrates the modes and directions of operation of the NBC in kidney proximal tubule cells, pancreatic duct cells, and hepatocytes.

CATION AND ANION SPECIFICITY

Cation and anion specificity of NBC has been studied in cortical BLM vesicles. Li^+ was the only other cation tested that showed any significant affinity for the cotransporter [22, 31]. However, the affinity for Li^+ was one

fifth of that for Na^+ [22]. In the presence of CO_2/HCO_3^- and the absence of initial pH and HCO_3^- gradients, the BLM vesicles loaded with Li^+ showed intracellular acidification [31]. This Li^+-dependent HCO_3^- efflux was inhibited in the presence of DIDS, consistent with the cotransport of Li^+ and HCO_3^- [31].

Anion specificity of NBC has been examined in kidney proximal tubule BLM. Media of various anions and varying $[CO_3^=]$ and $[HCO_3^-]$ were employed to examine the anion specificity and to evaluate the independent effects of $CO_3^=$ and HCO_3^- on ^{22}Na influx in BLM vesicles. In the presence of HCO_3^- , ^{22}Na influx was stimulated significantly in the presence of sulfite ($SO_3^=$), a carbonate analogue [22]. Increasing $[CO_3^=]$ at constant $[HCO_3^-]$ reduced the stimulation of ^{22}Na influx by $SO_3^=$, suggesting competition between $SO_3^=$ and $CO_3^=$. Sulfate ($SO_4^=$) or phosphate ($PO_4^=$) showed no affinity for NBC [22]. The question of whether NBC can accept hydroxyl (OH^-) in place of HCO_3^- was examined in rabbit renal BLM vesicles [32]. Imposing an inward pH gradient in the absence of HCO_3^- had no significant effect on DIDS-sensitive ^{22}Na uptake, indicating that NBC does not accept OH^-. In contrast to the kidney, the NBC in colon can accept OH^- in place of HCO_3^- [12].

INHIBITOR PROFILE

The inhibitor profile of NBC has been examined in several studies. The disulfonic stilbenes DIDS and dinitro-stilbene-disulfonate (DNDS) inhibit the cotransporter by competitive interaction with $CO_3^=$ [8]. The half-maximal inhibitor concentration (IC50) for DIDS was approximately 200 μmol/L [20], whereas for DNDS, it was approximately 400 μmol/L [8]. Harmaline inhibits the cotransporter by competitive interaction with Na^+ [22]. Of the other inhibitors tested, furosemide and bumetanide inhibited the NBC by >60% when added at a 1 mmol/L concentration [33].

Another interesting aspect of NBC is its interaction with the carbonic anhydrase inhibitor acetazolamide (ACTZ). Many studies have demonstrated that the carbonic anhydrase inhibitor ACTZ reduces the rate of H^+ secretion and HCO_3^- reabsorption in the proximal tubule by at least 80% [34–38]. Historically, major attention has been focused on the role of the luminal membrane carbonic anhydrase in catalyzing the breakdown of intratubular carbonic acid formed by the titration of filtered HCO_3^- with secreted H^+ [36, 39–41]. Two reports suggested that ACTZ prevents the exit of HCO_3^- from the proximal tubule cell when added to the peritubular fluid [42, 43]. Histochemical and immunocytochemical studies have demonstrated that carbonic anhydrase is localized not only at the luminal membrane, but also at the BLM and in the cytoplasm of the proximal tubule cell [44]. The interaction of ACTZ with NBC was examined in BLM vesicles [32]. These studies indicated that ACTZ can interact with NBC indirectly by blocking the carbonic anhydrase-mediated generation of HCO_3^- [32]. Based on these results, it was concluded that ACTZ can inhibit NBC by decreasing the substrate availability for this transporter at the inner surface of BLM [32].

pH_i SENSITIVITY

Regulation of NBC by pH_i has been studied in BLM vesicles by examining the effect of internal pH on HCO_3^--dependent $^{22}Na^+$ efflux [45]. Accordingly, BLM vesicles were pre-equilibrated with 1 mmol/L $^{22}Na^+$ at pH_i 6.8 to 8.0 and known concentrations of HCO_3^-. The vesicles were diluted 1:100 into Na^+-free solution at pH 7.4, and the net flux of $^{22}Na^+$ was assayed over five seconds. The results demonstrated that the net flux of Na^+ was significantly higher at pH_i 7.2 than pH_i 8.0, despite much higher $[HCO_3^-]$ and $[CO_3^=]$ at pH_i 8.0 [45]. Increasing the intravesicular concentration of HCO_3^- or $CO_3^=$ at constant pH_i did not inhibit ^{22}Na uptake [45], indicating that it was the pH_i rather than the substrate concentration that inhibited the cotransporter at alkaline pH. These results are consistent with the presence of a modifier site that inhibits the cotransporter at alkaline pH despite significant base concentration [45]. Further studies have shown that NBC activity also decreases in very acidic pH_i independent of $[HCO_3^-]$ [46]. Taken together, these results indicate that NBC is maximally functional around physiologic pH and is inhibited at acidic or alkaline pH_i [45, 46]. This is in contrast with the pH_i sensitivity of the other HCO_3^- extruding pathway, namely the Cl^-/HCO_3^- exchanger. This latter exchanger is stimulated at an alkaline pH_i [47]. The combination of modifier sites on the luminal NHE-3 [1–4, 48] and the basolateral NBC [45, 46] should help maintain intracellular pH in a narrow range with changes in extracellular pH.

REGULATION

Acute regulation

Acute acid-base disorders. Two frequent clinical conditions affecting pH homeostasis are metabolic acidosis and alkalosis. Primary metabolic acidosis, a condition marked by decreased serum $[HCO_3^-]$ and pH, has been shown to be associated with an increased ability of the proximal tubule to reabsorb HCO_3^- [49]. Primary metabolic alkalosis, a condition manifested by increased serum $[HCO_3^-]$ and pH, has been shown to be associated with a decreased ability of the proximal tubule to reabsorb HCO_3^- [50]. Studies that have evaluated the luminal NHE and basolateral NBC in animals with acid-base disorders suggest that the activity of the two transport processes are increased in metabolic acidosis and de-

creased in metabolic alkalosis [51, 52]. To determine whether the alteration in the activity of the NHE and NBC in acid-base disorders is due to systemic factors or is mediated locally at the level of the kidney, models of in vitro acidosis or alkalosis were used [53, 54]. Accordingly, rabbit proximal tubule suspensions were incubated in acidic (pH 7.0), control (pH 7.4), or alkaline (pH 8.0) media for 45 minutes and gassed with 5% CO_2. Brush border membrane (BBM) and BLM vesicles were then isolated and studied for NHE or NBC activity, respectively. The results showed that NBC is up-regulated in acute metabolic acidosis compared with control but remained unchanged in acute metabolic alkalosis (The absence of adaptive changes in NBC in response to acute metabolic alkalosis might suggest that proximal tubule cells are not well prepared to defend intracellular pH against alkalosis or that longer incubation time is needed to elicit an adaptive response) [53, 54]. NHE was up-regulated in acidosis and down-regulated in alkalosis [53, 54]. The alterations in the activities of these two transport processes were caused by changes in V_{max} [53, 54], consistent with either an increase in the number or turnover rate of the transporters. The cellular mechanisms responsible for these adaptive changes in in vitro acid-base disorders are presently unknown. Given the brief time of exposure of the tubule suspensions to acidic or alkaline pH, synthesis of new transport proteins seems unlikely. Indeed, cyclohexamide failed to prevent the adaptive increases in NHE and NBC in metabolic acidosis [53], lending support to this conclusion. Other possibilities include activation of currently inactive membrane proteins (phosphorylation) or incorporation of intracellular transporter proteins into the membrane (exocytosis). Staurosporin partially prevented the adaptive increases in NHE and NBC in acidic medium but had no effect on NHE or NBC in vesicles isolated from control or alkaline tubules [54]. These results suggest that protein kinase C (PKC) is partially responsible for the adaptive increases in NHE and NBC in metabolic acidosis.

Effect of parathyroid hormone, cAMP, and other kinases. The hyperparathyroid state is often accompanied by metabolic acidosis. This has been attributed to decreased acidification in the proximal tubule secondary to parathyroid hormone (PTH) effects. Several studies have shown that acutely administered PTH or cAMP inhibits HCO_3^- reabsorption in the proximal tubule [55–57]. These observations raised the possibility that this effect is mediated by decreased activity of the Na^+/H^+ exchanger in the luminal membrane of the renal proximal tubule. BBM vesicles isolated from suspensions of rabbit proximal tubules exposed to PTH or dibutyryl cAMP in vitro demonstrated a decrease in Na^+/H^+ exchange activity compared with control [58]. Studies in BLM vesicles that were pretreated with PTH showed decreased HCO_3^- dependent ^{22}Na influx consistent with inhibition of NBC [59]. Furthermore, this inhibitory effect was found to be mediated via cAMP [60].

Exposure to calmodulin decreased the HCO_3^--dependent ^{22}Na influx, whereas incubation with phorbol ester (a PKC activator) stimulated the HCO_3^--dependent ^{22}Na influx in BLM vesicles [60]. PKC was found to have long-term as well as short-term stimulatory effects on NBC. The long-term stimulatory effects of PKC on NBC (performed in cultured proximal tubule cells) were prevented by the protein synthesis inhibitors, actinomycin D, or cyclohexamide, whereas its short-term stimulatory effects were not [61]. Studies examining the role of PKC on luminal NHE are contradictory; although some studies have shown stimulation of luminal NHE, others have demonstrated inhibition of the exchanger by PKC [reviewed in 3, 4].

Effect of hormones. Effects of various hormones on NBC activity have been studied. Angiotensin II was found to increase luminal NHE and basolateral NBC directly and independently of one another in in vitro perfused rabbit kidney proximal tubule [62]. The stimulatory effect of angiotensin II (Ang II) on NBC and luminal NHE was acute and occurred in a matter of minutes [62]. In BLM vesicles isolated from rabbit kidney cortex, the addition of Ang II stimulated NBC activity, as assayed by HCO_3^--dependent ^{22}Na influx [63]. In the presence of pertussis toxin, Ang II failed to stimulate NBC, suggesting a role for G_i protein in mediating this effect [63]. Parathyroid hormone was found to inhibit the basolateral NBC, likely via protein kinase A pathways [60], as discussed earlier in this article. A recent report indicates that glucocorticoids up-regulate NBC activity in cultured proximal tubule cells from rabbit [64]. This effect, in contrast, depends on prolonged exposure to the hormone and requires the synthesis of new protein. In that regard, the effect of steroid is distinct from the effect of Ang II, which is acute.

Although the regulatory effect of renal sympathetic nerves on NBC has not been examined, it is plausible that because of vasoconstriction and the resulting enhancement of proximal tubule HCO_3^- reabsorption, this system enhances the activity of NBC.

Chronic regulation

Metabolic acidosis and alkalosis. Much evidence suggests that systemic pH may play a key role in the regulation of HCO_3^- reabsorption in the proximal tubule [1–4, 50]. For example, microperfusion studies of the rat proximal tubule have documented that systemic metabolic and respiratory acidosis increase, whereas metabolic alkalosis decreases luminal acidification [1–4, 50]. In vitro microperfusion studies in rat proximal tubule have indicated that luminal NHE and basolateral NBC are up-regulated in metabolic acidosis [52]. Akiba, Rocco, and

Warnock have shown that the luminal Na^+/H^+ exchanger and the basolateral NBC in rabbit kidney show parallel adaptation in metabolic acidosis and alkalosis [51]. In the case of metabolic acidosis, both NHE and NBC were up-regulated, and in metabolic alkalosis, both transporters were down-regulated. The stimulatory effect of metabolic acidosis or the inhibitory effect of metabolic alkalosis was due to alterations in the V_{max} of transporters [51]. Whether the primary transport process affected under these conditions is the luminal Na^+/H^+ exchanger or the peritubular NBC is unknown. If the adaptive changes primarily involve the NBC in the BLM, then cell pH and H^+ concentration should change accordingly. This, in turn, may affect the activity of the Na^+/H^+ exchanger by altering the H^+ gradient at the inner surface of the luminal membrane. Alternatively, alterations in the activity of luminal NHE can affect both cell pH and HCO_3^- concentration and subsequently alter NBC activity by affecting the availability of substrate for the cotransporter at the inner surface of the BLM. Adaptive parallel changes in NHE and NBC would prevent significant changes in cell pH and result in a pH_i that is held in a reasonably narrow range.

Respiratory acidosis and alkalosis. The effect of respiratory acidosis or respiratory alkalosis on NBC activity was examined in rabbits [65]. Respiratory acidosis or respiratory alkalosis was induced by exposing rabbits to 10% CO_2 (chronic hypercapnia) or 10% O_2/90% N_2 (chronic hypocapnia), respectively. BLM vesicles were then isolated from kidney cortices and assayed for NBC activity by ^{22}Na influx. BLM vesicles from rabbits with respiratory acidosis showed increased HCO_3^--dependent ^{22}Na influx compared with control, consistent with increased NBC activity [65]. BLM vesicles isolated from kidneys of rabbits with respiratory alkalosis showed decreased HCO_3^--dependent ^{22}Na influx compared with control, indicating decreased NBC activity. Na^+/H^+ exchange activity showed parallel adaptation with NBC; NHE activity increased in respiratory acidosis and decreased in respiratory alkalosis [65]. The inhibitory effect of respiratory alkalosis or stimulatory effect of respiratory acidosis on NBC or NHE was due to alterations in V_{max} and not Km of the respective transporters [65].

Adrenal corticosteroids. Adrenal corticosteroids are known to influence acid-base balance in humans and animals. For example, in patients with increased production of adrenal corticosteroids, as in Cushing syndrome, metabolic alkalosis is frequently present [66, 67]. Conversely, in conditions associated with decreased production of adrenal corticosteroids, as in Addison's disease, metabolic acidosis may develop [66, 67]. Whereas animals undergoing adrenalectomy show a decreased renal excretion of titratable acid and ammonium [68, 69], treatment with adrenal corticosteroids has been shown to stimulate renal acid secretion and ammonium production [70]. Although the direct and indirect effects of adrenal corticosteroids on renal acidification are complex and include mineralocorticoid effects in the distal nephron and effects of potassium in several tubule segments, glucocorticoids have been shown to enhance HCO_3^- reabsorption in the proximal tubule [70]. Kinsella et al have shown that the administration of the glucocorticoid dexamethasone to adrenalectomized rats increases the rate of Na^+/H^+ exchanger in BBM vesicles isolated from renal cortex [71–73]. These investigators found that dexamethasone altered the initial rate of Na^+ uptake by increasing the apparent V_{max} but not the apparent affinity of the exchanger for Na^+. The effect of adrenal steroids (glucocorticoids) on NBC activity is poorly studied. Incubation of primary cultures of the rabbit proximal tubule cells with dexamethasone increased Na-HCO_3^- cotransporter activity (as monitored by DIDS-sensitive HCO_3^--dependent ^{22}Na uptake). This effect was found to be mediated via a protein synthesis-dependent mechanism [65].

Potassium depletion. Potassium depletion has been associated with an increased ability of the renal tubules to reabsorb HCO_3^- [74–79]. This adaptive change in HCO_3^- reabsorption has been invoked as a possible mechanism involved in the pathogenesis of systemic metabolic alkalosis in disorders associated with potassium depletion [78]. Segmental analysis of the nephron indicates that potassium depletion is associated with increased HCO_3^- reabsorption by the proximal tubule, as well as the distal tubule [76–79].

Because the bulk of HCO_3^- reabsorption in the kidney proximal tubule results from the effects of NHE-3 and NBC-1 acting in series, studies were performed to examine the effect of potassium depletion on these two transporters. Rats were placed on a potassium-free diet for four weeks, and BBM and BLM vesicles were isolated from their kidney cortices in order to study NHE and NBC activities, respectively. ^{22}Na influx was measured to monitor the activities of transporters. The results indicated that potassium depletion induces a parallel increase in luminal NHE and basolateral NBC activity [79]. The increase in the activity of luminal NHE and basolateral NBC resulted from an increase in the V_{max} of the transporters. Recent studies have shown that NHE mRNA expression and protein abundance remained unchanged in KD animals [80], indicating that its functional up-regulation is a post-translational event. The effect of potassium depletion on NBC mRNA expression is distinct as will be discussed later.

CLONING, FUNCTIONAL EXPRESSION AND TISSUE DISTRIBUTION OF NBC ISOFORMS

As of the time of submission of this article, three human NBC isoforms (which we suggest be referred to as NBC-1, NBC-2, and NBC-3), two of which have

5′-end variants (NBC-1A and NBC-1B and NBC-3A and NBC-3B), have been released by GenBank. In addition, NBC1 species variants, *Caenorhabditis elegans*, rat, and mouse cDNA sequences have been released. NBC-1A, the first mammalian sequence to be reported, was cloned by Burnham et al from human kidney using a molecular probe, which was identified by searching for sequence similarities (to the anion exchangers) in the "expressed sequence tag" database section of GenBank [23].

The amphibian (*Ambystoma tigrinum*) kidney NBC, which was cloned by expression in *Xenopus* oocytes, is designated as NBC-1B [24]. Both human and ambystoma open reading frames are of similar molecular weight (1035 amino acids), but differ in message size. The human kidney NBC-1 cDNA was 7.6 kb in length, which is consistent with the size of the human mRNA as it appears on a Northern blot. However, the amphibian kidney mRNA is much shorter, at approximately 4.2 kb. The difference appears to be the result of an extensive 3′ noncoding region in the human NBC-1 kidney cDNA. The amino acid sequences of the two species are 80% identical.

Burnham et al reported that the human kidney NBC-1 gave a strong hybridization signal on a human pancreas Northern blot, suggesting that it is also expressed there [23]. Abuladze et al cloned a cDNA from human pancreas that is identical to human kidney NBC-1 except that it has a unique 5′ end including 85 amino acids of open reading frame, which replace the first 41 amino acids of the human kidney sequence [28]. When the unique 5′ ends of the two variants were used to probe multiple-tissue Northern blots, the kidney variant hybridized only with kidney mRNA, whereas the pancreas variant gave strong signals with prostate, colon, stomach, thyroid, brain, and spinal chord, as well as pancreas mRNA [28]. The junction at which the kidney-specific variant diverges from the more widely expressed variant from pancreas is likely to be at an intron–exon boundary, and alternative promoters or splicing may be involved in the generation of the differences. Because the two amino acid sequences differ by less that 1% overall, the two variants are likely to be encoded by the same gene.

More recently, the pancreas variant has been cloned from a human heart cDNA library [81]. The hybridization signal of heart NBC-1 mRNA on a Northern blot is vanishingly faint compared with pancreas, suggesting that other NBC isoforms may be responsible for the majority of both the electroneutral [82] as well as the electrogenic [83] Na^+:HCO_3^- cotransport, which has been described functionally in the heart.

It is questionable whether the term "isoform" is correct in referring to the distinction between the kidney variant reported by Burnham et al [23], and the more widely expressed variant subsequently reported by Abuladze et al [28], because the term "isoform" is usually restricted to the product of a unique gene rather than to the result of a variation (most likely) resulting from alternate splicing of a single gene. The terminologies such as "hhNBC" for "human heart NBC" [81] may therefore be more confusing than helpful, as this variant was neither first identified in human heart nor is it likely to be the predominant sodium bicarbonate cotransporter present in the heart. Other isoforms (discussed later in this article) appear more likely to be physiologically relevant under normal conditions in the heart.

This issue is particularly relevant because GenBank has assigned the designation "SLC4A" for "SoLute Carrier family 4 Anion exchanger" to the family of bicarbonate transporters that includes the anion exchangers as well as the NBCs. GenBank has designated AE1, 2, and 3 as "SLC4A1, 2, and 3," however, NBC-1a (the kidney specific variant) has been assigned the designation SLC4A4, whereas the more widely expressed variant NBC-1b (first found in pancreas) has been assigned the designation "SLC4A5." The two variants do not warrant separate locus identification designations.

NBC-2, which is now designated SLC4A6, is a unique isoform with 53% identity with NBC-1. NBC-2 was identified by searching an EST database for sequences similar to AE1 and isolated from a human retina cDNA library by Ishibashi, Sasaki, and Fumiaki [84]. NBC-2 is widely expressed, being found in testis, spleen, colon, small intestine, ovary, thymus, prostate, skeletal muscle, heart, kidney, stomach, trachea, and bone marrow. It appears to be absent from pancreas and liver. Two variants of NBC-2 appear to be expressed in a tissue-dependent manner, one of approximately 8 kb, which predominates in human retina, and one of approximately 5 kb, which predominates in human testis. Based on the fact that NBC-2 mRNA expression is highly abundant in PS120 cells (whereas other NBC isoforms are absent) and based on the observation that Na-dependent Cl^-/HCO_3^- exchanger is the only Na-dependent HCO_3^- cotransport system in these cells, it was proposed that NBC-2 is likely the Na^+-dependent Cl^-/HCO_3^- exchanger (abstract; Burnham and Soleimani, *J Am Soc Nephrol* 9:3A, 1998). A definite answer with respect to the functional identity of NBC-2 should come from transfection or expression studies.

NBC-3, which has not yet been assigned a solute carrier family 4 designation, is the third isoform to be identified [85]. It appears to have three transcripts on Northern blots, which are expressed in a tissue-dependent manner [85]. A large, approximately 9 kb transcript is expressed in brain, spinal chord, and adrenal gland [85]. An intermediate transcript of approximately 4.5 kb is widely expressed in brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, stomach, thyroid, spinal chord, lymph node, trachea, adrenal gland, and bone marrow [85]. A small transcript (approximately 3 kb) appears in neuroneal

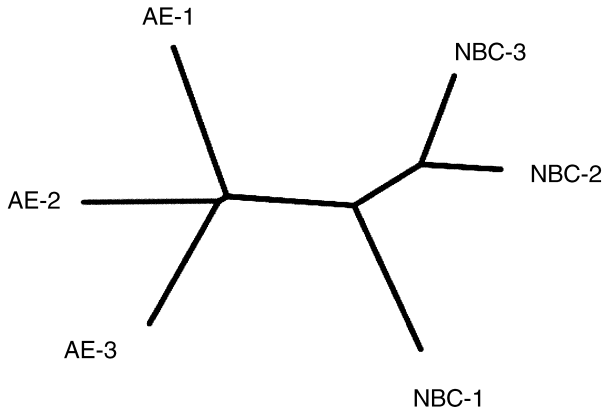

Fig. 2. A phenotypic dendrogram illustrating the evolutionary relationships among the AE/NBC superfamily. Branch lengths are proportional to the evolutionary distance calculated using a "blossom" matrix. Branch lengths of variants (for example, SLC4A4 vs. SLC4A5 or NBC1a vs. NBC1b) were not of sufficient length to be visible. Definitions are: NBC, Na^+:nHCO_3^- cotransporter; AE, anion exchanger.

tissue. Two variants of NBC-3 have been released by GenBank (accession numbers AB018282 and AF069512). The first was identified from randomly sequenced cDNA clones from a human brain library by Nagase et al [86]. The second to be released (Grichtchenko et al, unpublished data) contains an alternate 5′ end, but is identical in other respects. Tissue distribution studies on each of the two NBC-3 variants have not been reported. Overall, NBC-3 has an amino acid sequence that is 56% identical with NBC-1 and 76% identical with NBC-2.

A phenotypic dendrogram illustrating the relationships of the bicarbonate transporter/exchanger family is shown in Figure 2. The anion exchanger part of the family appears to have branched off from a common ancestor nearly simultaneously, whereas NBC-1 is derived from a form that was intermediate between the AEs and NBC-2 and NBC-3. A multiple sequence alignment of NBC-1, NBC-2, and NBC-3 is shown in Figure 3.

MOLECULAR REGULATION OF NBC ISOFORMS

Acid-base disorders

Recent studies support the conclusion that NBC-1 is the proximal tubule basolateral NBC [25–27]. To understand the regulation of NBC-1 better, the expression of NBC-1 was studied under three models of acid-base imbalance: chloride-depletion alkalosis, metabolic acidosis, and bicarbonate loading [25]. Chloride-depletion alkalosis was induced by intraperitoneal dialysis against a chloride-free, high bicarbonate solution for 30 minutes. Metabolic acidosis was induced by placing rats on NH_4Cl (280 mmol/L, added to their drinking water) for four days. For bicarbonate loading, $NaHCO_3$ (280 mmol/L) was added to the rats' drinking water. NBC-1 mRNA was decreased in bicarbonate loading, indicating reduced HCO_3^- reabsorption in kidney proximal tubule [25]. This should result in increased bicarbonate excretion and the maintenance of normal acid-base balance. NBC-1 mRNA did not change in metabolic acidosis or alkalosis [25], suggesting that alterations in cotransport activity in kidney proximal tubule under these two circumstances [5–8] is a post-transcriptional event.

Studies in cultured mouse inner medullary collecting duct (mIMCD-3) cells showed high levels of NBC-1 and low levels of NBC-3 mRNA expression [85]. NBC-1 and NBC-3 were differentially regulated by sublethal acid stress [85]. Subjecting the cells to severe acid stress decreased the mRNA expression of NBC-1 by approximately 90%, but increased the mRNA expression of NBC-3 by approximately 5.5-fold [85]. Increased expression of NBC-3 was associated with enhanced Na-dependent HCO_3^- cotransport activity by approximately sevenfold [85]. It was proposed that NBC-3 was likely involved in cell pH regulation by transporting HCO_3^- from blood to the cell, and its enhanced expression in severe acid stress played an important role in cell survival by mediating the influx of HCO_3^- into the cells [85].

Potassium depletion

Based on functional studies indicating increased activity of basolateral NBC in K^+-depleted rats [79], an attempt was made to examine the effect of potassium depletion on NBC-1 mRNA expression and activity. Rats were placed on a K^+-free diet and sacrificed after 2, 3, 6, or 21 days. Tubular suspensions were isolated from proximal tubule, medullary thick ascending limb, and inner medullary collecting duct, and were assayed for the expression and activity of NBC-1. Northern hybridizations indicated that NBC-1 mRNA expression in proximal tubule increased as early as 72 hours after potassium deprivation and remained elevated at 21 days [87]. Enhanced expression of NBC-1 was associated with increased NBC activity in proximal tubule suspensions [87]. Interestingly, NBC-1 expression was induced in medullary thick ascending limb and inner medullary collecting duct of potassium deprived rats [87]. These results indicate that potassium deprivation increases HCO_3^- reabsorption in proximal tubule by enhancing NBC-1 expression and activity. The results further indicate that by inducing NBC-1 expression in mTAL and IMCD, potassium deprivation enhances HCO_3^- reabsorption in these nephron segments. It becomes apparent that upregulation of NBC-1 is an early event and precedes the onset of hypokalemia [87], indicating that the signal responsible for enhanced NBC-1 expression is likely activated via intracellular potassium depletion rather than hypokalemia.

Glucocorticoid excess

The effect of glucocorticoids on NBC-1 was recently studied [88]. Rats were injected subcutaneously with either dexamethasone (100 μg day) or deoxycorticosterone acetate (DOCA; 30 mg/kg), potent glucocorticoid or mineralocorticoid analogues, respectively. Animals were sacrificed after five days, and NBC-1 mRNA expression and activity were measured in proximal tubule suspensions. Northern hybridizations indicated that dexamethasone treatment enhanced NBC-1 mRNA expression and activity in proximal tubule of rats treated with dexamethasone [88]. Treatment of rats with DOCA did not alter the expression of NBC-1 [88]. These results indicate that glucocorticoids but not mineralocorticoids enhance both mRNA expression and functional activity of renal proximal tubule NBC-1. Enhanced NBC activity could result in increased HCO_3^- reabsorption in proximal tubule in pathophysiologic states associated with increased glucocorticoid production.

Cystic fibrosis

Cystic fibrosis remains a major healthcare problem worldwide [89]. An autosomal recessive disease, cystic fibrosis results from the mutational inactivation of a cAMP-sensitive Cl^- channel [cystic fibrosis transmembrane conductance regulator (CFTR)] with resultant impairments in the respiratory, pancreatic, hepatobiliary, and genitourinary systems [89]. With regard to the pancreas, pancreatic dysfunction is felt to result primarily from impairment of secretin-stimulated ductal Cl^- and HCO_3^- secretion [89]. Experimental and histopathologic evidence suggests that the reduction in secretin-stimulated HCO_3^- secretion from pancreatic duct epithelial cells alters intraductal pH sufficiently to precipitate proteins secreted from acinar cells, resulting in protein plugs and disruption of vesicular trafficking in the acinar cell's apical domain [90]. Taken together, these alterations lead to pancreatic fibrosis and insufficiency in 80% of cystic fibrosis cases [90]. Attempts toward understanding or correcting this HCO_3^- secretion defect have been hampered by a lack of knowledge regarding the cellular and molecular mechanisms mediating HCO_3^- transport in these cells. The currently accepted model of pancreatic ductal HCO_3^- secretion suggests that (*1*) intracellular HCO_3^- accumulates because of basolateral diffusion of CO_2 and subsequent action of carbonic anhydrase; (*2*) G-protein–coupled receptors activate cAMP-sensitive CFTR; and (*3*) resultant increases in luminal Cl^- drives a Cl^-/HCO_3^- exchanger [30]. However, this model is inconsistent with recent data demonstrating the Na^+ dependence of ductal HCO_3^- uptake at the BLMs and lack of inhibition of secretin-stimulated HCO_3^- secretion in the absence of luminal Cl^- [91].

A recent study demonstrated that HCO_3^- enters duct cells (from blood) via the basolateral electrogenically-driven Na^+:HCO_3^- cotransporter, NBC-1 [29]. It was further illustrated that cAMP, which mediates the stimulatory effect of secretin on HCO_3^- secretion, potentiated Na^+:HCO_3^- cotransport only in cells expressing functional CFTR. This stimulatory effect of cAMP was blocked under voltage-clamped conditions (that is, in the presence of a potassium ionophore and high extracellular K^+), indicating that the stimulation of Na^+:HCO_3^- cotransport by cAMP is due to the generation of a favorable electrical potential as a result of membrane depolarization by Cl^- secreting CFTR [29]. Based on these studies, it was proposed that the driving force for HCO_3^- entry into pancreatic duct cells in the agonist-stimulated state is activation of the apical CFTR in response to intracellular cAMP [29]. The observed depolarization of cell membrane potential (which results from Cl^- exit at the luminal membrane via CFTR) stimulates the entry of HCO_3^- via the basolateral electrogenically-driven Na^+:HCO_3^- cotransporter, NBC-1. HCO_3^- is then secreted into the duct lumen predominantly via a process other than Cl^-/HCO_3^- exchanger. It was further proposed that the defect in HCO_3^- secretion in response to secretin (which acts via cAMP) in patients with cystic fibrosis is due to lack of entry of HCO_3^- at the BLM (caused by a lack of depolarization of the cell membrane and therefore the absence of a driving force for electrogenic Na^+:HCO_3^- cotransport). Accordingly, the following HCO_3^- transport model has been proposed in the pancreatic duct cells (Fig. 4). According to this model, secretin increases intracellular cAMP, which then results in the activation of CFTR and secretion of Cl^-, leading to depolarization of both luminal and BLMs. The depolarization of the BLM increases the driving force for NBC and, as a result, enhances HCO_3^- entry into the duct cells. HCO_3^- will then be secreted at the apical membrane (mostly via a poorly defined mechanism at the present and partially via an apical Cl^-/HCO_3^- exchanger). These findings have important ramifications regarding our understanding of the physiologic role of CFTR in these cells and provide important insight into the pancreatic pathophysiology of cystic fibrosis.

Based on the important role of NBCs in HCO_3^- transport and cell pH regulation in kidney, pancreas, and cardiac cells, it is plausible that dysregulation of NBCs may play a role in the pathogenesis of proximal renal tubular acidosis, pancreatitis, and cardiac dysfunction, respectively. Future studies in genetically engineered mice deficient in NBCs should answer questions regarding the role of these transporters in electrolyte and acid-base homeostasis in kidney, pancreas, and other organs.

In conclusion, NBCs are expressed in various nephron segments and are essential to acid-base and electrolyte homeostasis. NBC-1 plays an important role in HCO_3^- reabsorption in the proximal tubule, whereas NBC-3 is

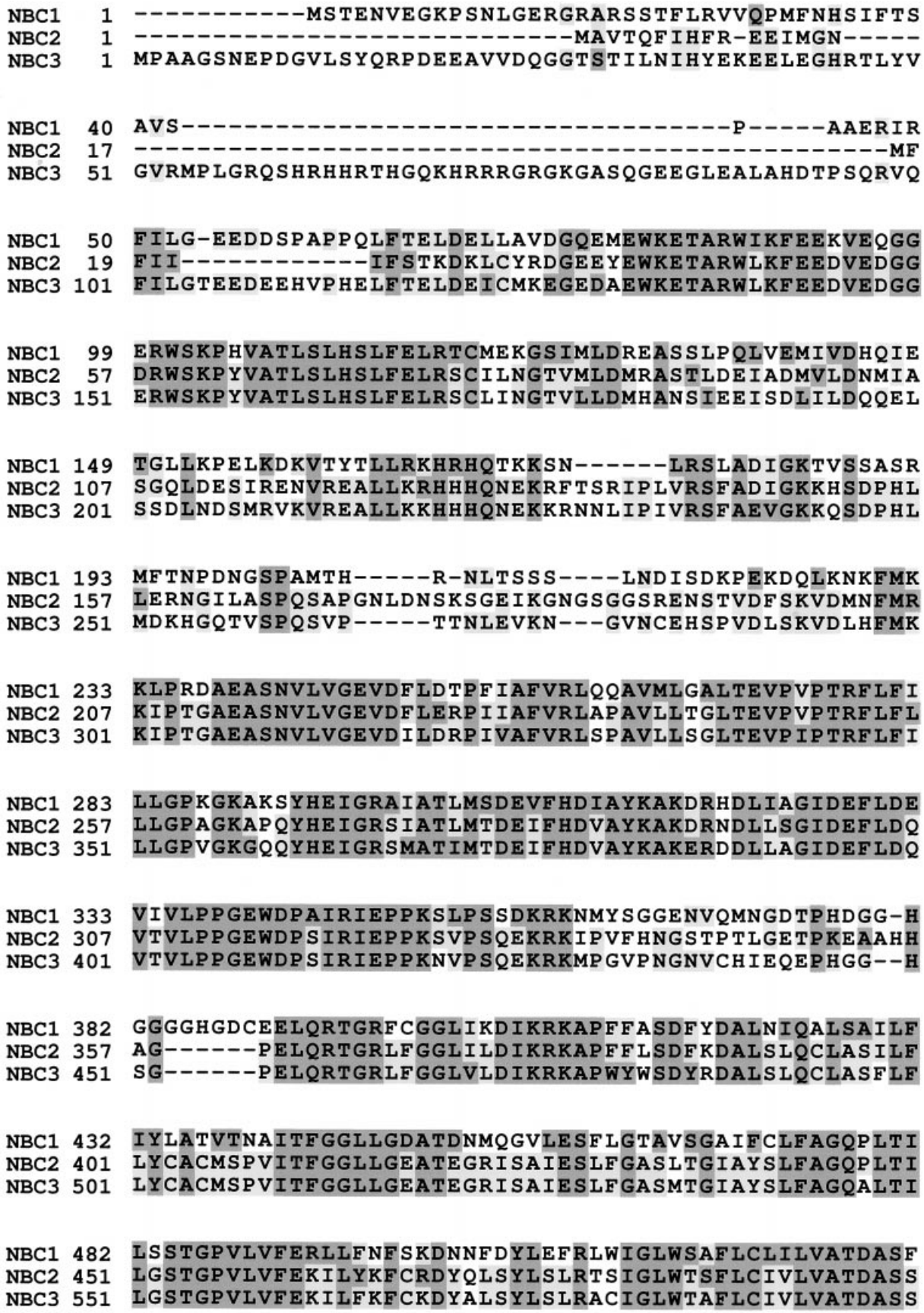

Fig. 3. A multiple sequence alignment of NBC-1, NBC-1, and NBC-3. Sequences were aligned using the Molecular Biology Workshop Website from the University of Illinois. The Website has online implementations of Clutal-W and MSAShade for sequence alignment and shading, respectively, as well as a phylogenetic dendrogram capabilities.

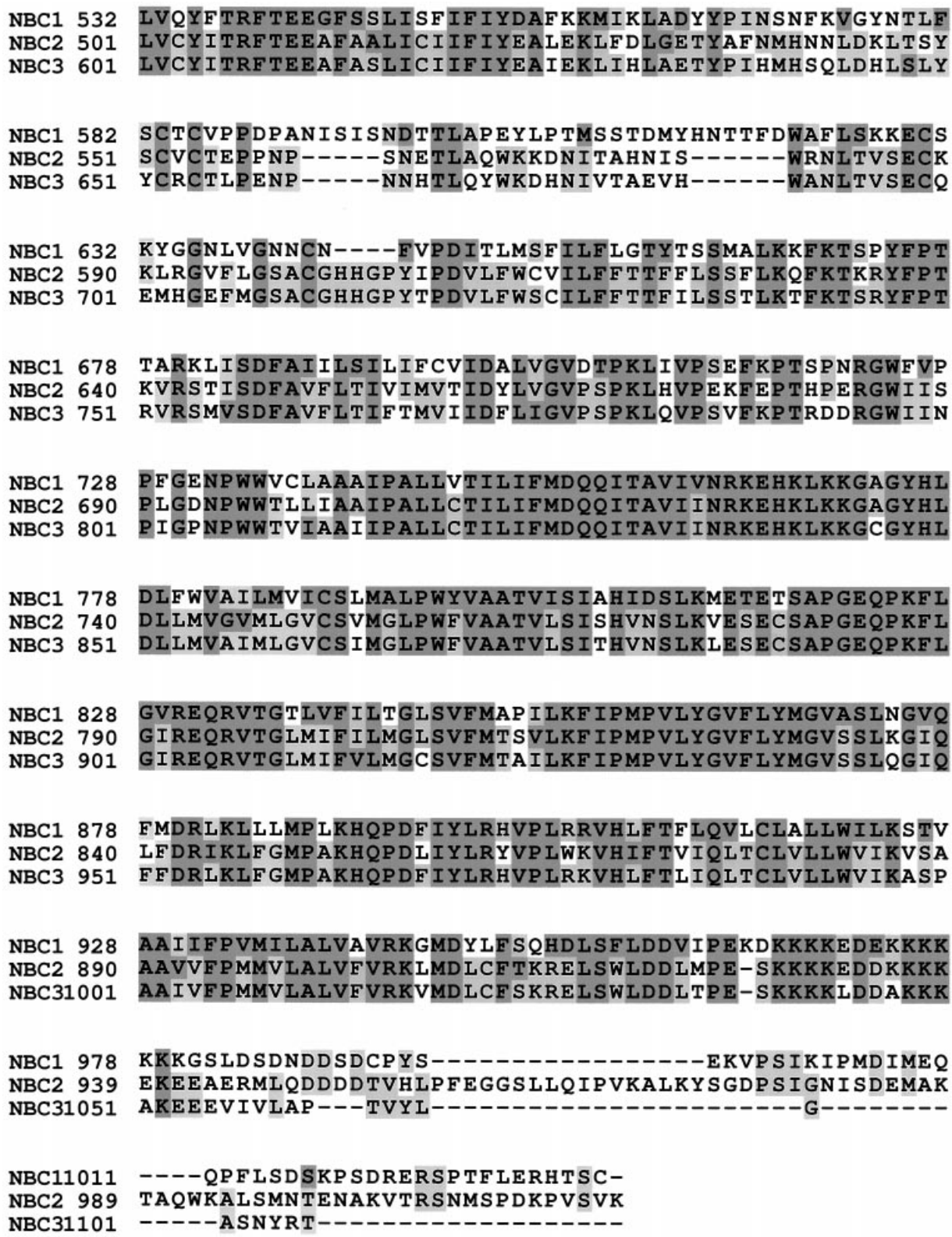

Fig. 3. Continued.

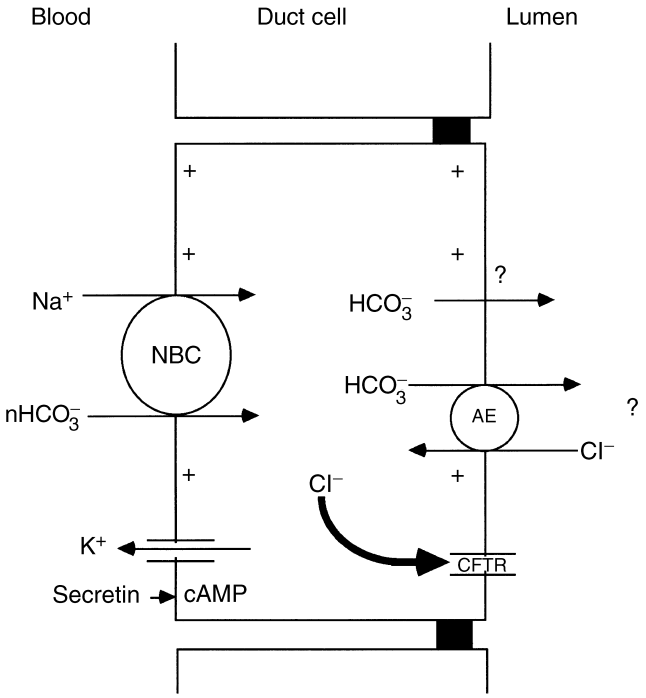

Fig. 4. Proposed model of HCO_3^- secretion in the pancreatic duct cells. Activation of cystic fibrosis transmembrane conductance regulator (CFTR) depolarizes the basolateral membrane potential, which, in turn, stimulates the electrogenic Na^+:$nHCO_3^-$ cotransporter (NBC-1). The HCO_3^- that is thus entered is secreted at the apical membrane predominantly via a transporter other than Cl^-/HCO_3^- exchanger. The addition sign denotes the depolarized membrane potential.

predominantly expressed in the inner medulla. The nephron segment distribution of NBC-1 is species specific; in rats, NBC-1 is predominantly localized to the proximal tubule, whereas in mouse, it has a wider tissue distribution and is expressed in cortex, outer, and inner medulla (American Society of Nephrology abstracts, 1997). NBC-2 is likely a Na-dependent Cl^-/HCO_3^- exchanger and may be involved in cell pH regulation. More studies are needed to examine the functional identity of NBC-2 and its nephron segment distribution and regulation.

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